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RELATION OF CULTIVATION TO DEPLETION OF ROOT RESERVES IN EUROPEAN BINDWEED AT DIFFERENT SOIL HORIZONS [1]

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INTRODUCTION

It is well known that the roots of the European bindweed (*Convolvulus arvensis L.*) penetrate to considerable depths in the soil and that control by cultivation is effected by depletion of the root reserves. A number of investigators, including Bakke, Gaessler, and Loomis (*2*),[2] Frazier, as cited by Timmons (*9*), Bioletti (*5*), and Kiesselbach et al. (*7*), have reported roots at depths of 15 to 25 feet. Timmons (*9*) found very few roots below 6 feet in the dry upland soil at Hays, Kans.

Most root reserve studies, including those reported by Bakke et al. (*2*), Barr (*3, 4*), and others, have been limited for the most part to the upper foot or 2 feet of soil. Obviously, such studies with European bindweed take into account only a part of the entire root system. It is important to know whether the roots in the upper soil layers are representative of the whole since excavation of all roots of such a deep-rooted plant is virtually impossible and removal of even a major portion is an arduous task. The primary objective of the work reported in this paper was to obtain this information. Second objective was to secure such data as might at the same time be obtained on the concentration and rate of depletion of reserves, and the death of the bindweed roots at various soil levels.

MATERIAL AND METHODS

COLLECTING THE ROOTS

The problem was approached by excavating the roots at various intervals on plots that were being cultivated for bindweed eradication at the Cherokee State Hospital farm, Cherokee, Iowa. The roots were kept separately for each soil level and analyzed for dry-matter content, reducing sugars, starch equivalent, and nitrogen.

The field on which the experiments were carried out is reported by employees of the State hospital to have been infested with bindweed for more than 20 years. The area of bindweed infestation is extremely variable, however, suggesting that most of the bindweed came by gradual spread from a few original patches rather than by seed, as in

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[2] Italic numbers in parentheses refer to Literature Cited, p. 147.

seed grain, for example. At the inception of the experimental work in 1936, the area was carefully surveyed and the infestation, based on visual inspection of the above-ground growth, recorded. Only the most heavily and uniformly infested areas were used in these studies. Three areas designated as plots 42, 72, and 142, each 4 by 2 rods, were chosen for the work and from these excavations were made and bindweed roots removed for analysis.

These plots were chosen because they were uniformly infested and appeared to be suitable for the purpose and because they were available at the time the excavations could be made. They were not intended as replicates of each other nor were they chosen to compare the root reserves under different treatments, though it is possible that some useful information might be obtained by comparing the results from the three plots.

The area had been used for general farming before the plots were laid out in 1936. Plot 42 was fallowed in 1937; i. e., for 1 year before the first excavations were made. The others prior to the first sampling date were handled no differently than might be expected on a well-managed farm, though somewhat better than on many bindweed-infested farms. The first excavations were made on plots 42 and 72 in May 1938 and on plot 142 in May 1939. After the first excavations the remainder of each plot was cultivated at frequent intervals (2 weeks or less) for the duration of the study. Sampling of plots 42 and 142 was continued for 2 years and sampling of plot 72 for 3 years. At the end of these periods practically all the bindweeds were dead.

Three or more excavations were made each year until the bindweeds on the unexcavated portions of the plots were dead or until it was no longer possible to get sufficient roots for analysis. The first sampling in each area was made at about the time the leaf-bearing shoots first appeared above the ground, the second in late June or early July at the approximate date of maximum above-ground growth, and the last in October or early November when growth had ceased. In 1939 and 1940, excavations were made in August also.

All excavations were 5x5 feet and as deep as roots were present in sufficient quantities to provide material for a chemical analysis from each soil level, i. e., about 4 gm. of dry roots per 25 cubic feet of soil. This depth was 8 feet for plots 42 and 72, and 6 feet for plot 142 for the first sample. At some of the later dates the quantity of roots at the lower depths was not sufficient for analysis.

The locations of the excavations made in each plot were selected at random, except that each excavation after the first was made at a sufficient distance from the earlier ones to avoid any effects on the bindweed that might have resulted from tramping and the disposition of material from the hole. The soil was removed in 1-foot layers, and during the process of removal the bindweed roots were separated by hand and placed in a 2-gallon milk can which was kept covered with a wet towel to prevent evaporation. Only living roots or living portions of roots were taken. These roots were then carefully washed to remove adhering soil particles, dried with toweling or cheesecloth until the surface was dry, weighed, subjected to live steam for 10 minutes to kill them, and immediately placed in a Hixon and Bakke field dehydrator (*6*) where they were left until completely dry. The dry material was later sent to the plant chemistry subsection, Iowa State College, for analysis.

CHEMICAL ANALYSIS

The samples as received were nearly, if not completely, air-dry. They were ground in a Wiley mill through a 40-mesh screen. The moisture content of the air-dried sample was determined by drying in a vacuum oven held at 70° C. and 25 to 28 inches of mercury for 14 hours. If the samples were less than 10 gm. in weight, a micro mill was used. A normal, complete analysis required 6 gm. of dry material. All determinations were made in duplicate. A 1-gm. sample sufficed for both the moisture and the nitrogen determinations, while 2 gm. were sufficient for both the sugar and the starch.

The sample from the moisture determination was used directly for the Kjeldahl (*1*) determination of nitrogen. The sample from the weighing bottle was conveniently transferred directly to a filter paper and inserted into a Kjeldahl flask. One mercury pill (consisting of 0.7 gm. mercuric oxide) was placed in each flask, as a catalyst. The remaining steps in the procedure are given in the Official Methods of Analysis (*1*).

The sugars were extracted from a 2-gm. aliquot sample of the ground material, being transferred to a Bailey-Walker thimble fitted with a 9-cm. filter paper; 30 cc. of 80-percent alcohol was added and the sample was extracted on a Baily-Walker extraction apparatus for 12 hours, cleared with neutral lead acetate, and deleaded with solid sodium oxalate, freed from alcohol, hydrolyzed with HCl at 25° C., and the total reducing sugars determined by the Shaffer and Hartmann method (*8*).

The residue from the sugar extraction was transferred to a 400-cc. beaker and gelatinized by autoclaving at 120° C. for 15 minutes at 15 pounds pressure. Malt diastase was used to digest the starch, and the filtrate was hydrolyzed with HCl without clearing. The reducing value of neutralized extract was also determined as before by the Shaffer-Hartmann method. The resulting values are here referred to as starch equivalent.

The nitrogen, reducing sugars, and starch-equivalent determinations are reported herein as percentage of dry matter. The reducing sugars and starch-equivalent determinations were added together and are here referred to as total "available" carbohydrates, though it is recognized that all the materials so designated may not be available to the plant.

EXPERIMENTAL RESULTS

The quantity of bindweed roots per cubic foot of soil, the percentage of dry matter, the total available carbohydrates expressed as percentage of the dry weight of roots, and per cubic foot of soil for plot 42 are presented in table 1. Similar data for plots 72 and 142 are given in tables 2 and 3. The total available carbohydrates and the quantity of roots are shown graphically in figures 1 and 2. The nitrogen content expressed as percentage of the dry matter, and the proportion of reducing sugars and starch equivalent varied considerably according to the depth of the roots and the time of sampling. Since they appeared to have no important bearing on the objectives of this study, the data are omitted in the interests of brevity. The nitrogen content of the roots of plot 142 at all depths and all sampling dates was from about 1.5 to 4 times greater than on plots 42 and 72. A reviewer of this paper suggests that the rapidity with which the bindweed on plot 142

were killed by cultivation may have been due to this fact. This cannot be denied although it would appear that the smaller quantity of roots and of available carbohydrates at the outset is an equally probable explanation.

The relative concentration of total available carbohydrates in different soil levels, and the rate of depletion of these carbohydrates and of roots, as may most readily be seen in figures 1 and 2, are substan-

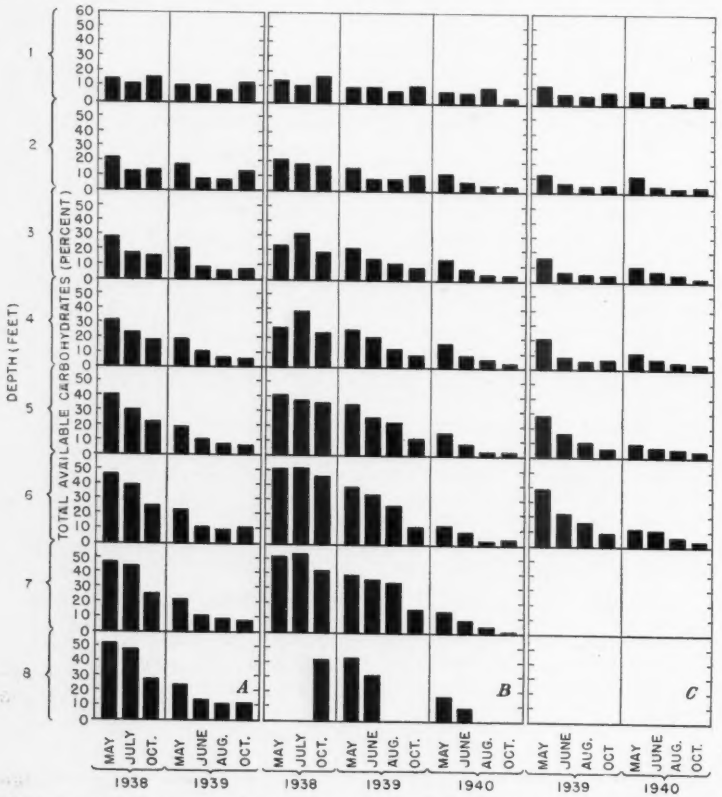

FIGURE 1.—Total available carbohydrates expressed as percentage of the dry weight in the roots of European bindweed at various depths in the soil: *A*, Plot 42; *B*, plot 72; and *C*, plot 142, at Cherokee, Iowa, 1938, 1939, and 1940.

tially alike for all three plots. Hence the results for all of them can conveniently be discussed at one time.

Perhaps the most significant facts shown by these data are the high concentration of available carbohydrates in the lower soil levels (fig. 1) and the concentration of roots in the upper levels (fig. 2). The former increases and the latter decreases with depth. The rate of change in root quantity is greater than that for percentage of available carbohydrates and consequently the quantity of the latter (tables 1 to 3)

expressed as grams per cubic foot of soil is much greater in the surface or near-surface levels.

On the first sampling date the surface foot of the freshly excavated roots contained 24.6 percent of all the roots in plot 42, 27.0 percent in plot 72, and 24.7 percent in plot 142. Corresponding figures for the upper 2 feet are 47.2, 49.8, and 50.3 percent. The values for

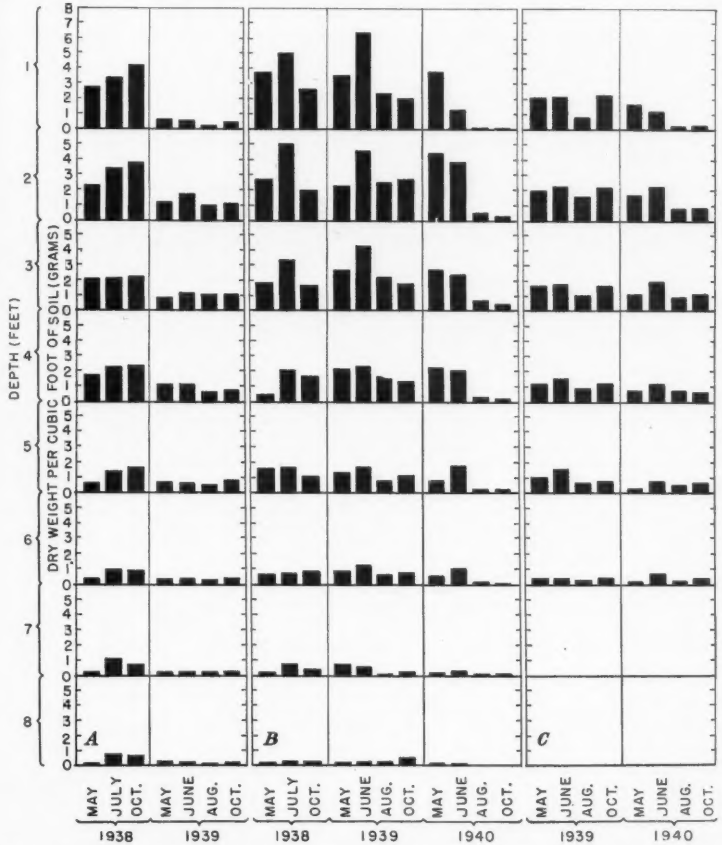

FIGURE 2.—Dry weight of roots of European bindweed in grams per cubic foot of soil at various depths in the soil: *A*, Plot 42; *B*, plot 72; and *C*, plot 142, at Cherokee, Iowa, 1938, 1939, and 1940.

available carbohydrates in the upper foot of the three plots were 18.4, 14.1, and 21.6 percent and for the upper 2 feet 32.6, 34.2, and 46.2 percent. It would appear, therefore, that in these plots sampling to a depth of 2 feet would have included about 50 percent of the roots and one-third or more of the available carbohydrates. It is probable that had the first samples been taken at a different time of the year or on plots so handled that the bindweed had had a better

opportunity to grow, there would have been a larger proportion of roots and of the available carbohydrates in the upper foot or 2 feet of soil.

There was a constant decrease in quantity of roots and in percentage and quantity of carbohydrates with time, accompanied as in this case

TABLE 1.—*Quantity of roots and dry matter, and total available carbohydrates in the roots of European bindweed in plot 42*

Depth (feet)	1938			1939			
	May	July	Oct.	May	June	Aug.	Oct.
QUANTITY OF ROOTS (GRAMS DRY WEIGHT PER CUBIC FOOT OF SOIL							
1	2.77	3.46	4.26	0.80	0.62	0.30	0.55
2	2.38	3.48	3.88	1.31	1.82	1.02	1.15
3	2.08	2.20	2.34	.97	1.19	1.04	1.03
4	1.75	2.32	2.38	1.09	1.10	.64	.81
5	.63	1.45	1.64	.71	.58	.47	.92
6	.37	.99	.96	.37	.39	.28	.42
7	.27	1.02	.74	.27	.28	.24	.29
8	.15	.80	.76	.29	.26	.14	.24
Total	10.40	15.72	16.96	5.81	6.24	4.13	5.41
DRY MATTER (PERCENTAGE OF THE WEIGHT OF FRESH ROOTS)							
1	12.0	13.7	20.6	17.3	12.4	13.4	17.6
2	17.5	20.3	21.7	20.3	20.4	18.1	19.6
3	24.0	18.3	25.4	20.4	18.5	17.5	20.6
4	25.7	17.7	25.3	17.9	16.2	15.8	19.1
5	23.5	17.3	16.8	18.6	14.8	15.1	17.1
6	21.8	17.0	14.7	16.7	13.8	12.8	15.6
7	24.7	18.5	13.4	15.3	12.5	12.8	14.2
8	21.5	22.5	13.5	14.7	13.3	12.3	12.0
TOTAL AVAILABLE CARBOHYDRATES (PERCENTAGE OF DRY WEIGHT OF ROOTS)							
1	15.56	13.81	17.00	10.17	10.21	8.45	13.89
2	21.40	12.41	14.14	17.31	7.28	7.26	13.77
3	29.00	17.35	15.31	21.23	8.95	6.50	6.93
4	30.13	22.95	18.05	18.40	10.07	6.91	6.86
5	41.83	31.58	22.37	19.80	10.59	7.52	6.87
6	47.05	39.66	25.97	23.78	10.29	9.98	10.14
7	47.54	44.44	25.09	22.13	10.47	8.91	7.88
8	51.56	48.80	27.24	24.56	13.41	10.26	10.42
TOTAL AVAILABLE CARBOHYDRATES (GRAMS PER CUBIC FOOT OF SOIL)							
1	0.431	0.478	0.724	0.081	0.063	0.025	0.076
2	.509	.432	.549	.227	.132	.074	.213
3	.603	.382	.358	.206	.107	.068	.084
4	.527	.532	.430	.201	.110	.034	.056
5	.263	.458	.167	.141	.061	.035	.063
6	.174	.393	.249	.088	.041	.027	.033
7	.127	.453	.186	.060	.029	.021	.023
8	.077	.390	.207	.071	.035	.014	.025
Total	2.711	3.518	2.870	1.075	.578	.298	.573

by cultivation. The first effect of the cultivation was to reduce the concentration of carbohydrates. This decrease continued in plot 42 until a low point was reached several weeks or months before the bindweed plants were killed. There were indications of a similar relation in plots 72 and 142, but the evidence is less conclusive. For a while there appeared to be no decrease in root weight other than could be ac-

counted for by loss of carbohydrates. For example, on plot 42 the quantity of roots in the excavation made in October 1938 was actually greater than in the one made in May, a result, no doubt, of variability in bindweed infestation. In plots 72 and 142 there were no appreciable differences in the quantity of roots in May and in October of the

TABLE 2.—*Quantity of roots and dry matter, and total available carbohydrates in the roots of European bindweed in plot 72*

QUANTITY OF ROOTS (GRAMS PER CUBIC FOOT OF SOIL

Depth (feet)	1938			1939				1940			
	May	July	Oct.	May	June	Aug.	Oct.	May	June	Aug.	Oct.
1	3.82	5.08	2.57	3.51	6.40	2.46	2.10	3.94	1.37	0.03	0.03
2	2.81	5.10	2.07	2.30	4.58	2.50	2.74	4.40	3.80	.52	.41
3	1.98	3.47	1.67	2.87	4.30	2.30	1.90	2.79	2.48	.64	.45
4	1.00	2.19	1.71	2.22	2.40	1.61	1.40	2.27	2.05	.44	.26
5	1.68	1.71	1.18	1.42	1.78	.90	1.21	.90	1.80	.29	.24
6	.65	.68	.80	.88	1.24	.59	.84	.50	1.00	.21	.11
7	.21	.78	.42	.78	.63	.19	.25	.33	.38	.09	.09
8	.18	.35	.35	.25	.28	.28	.55	.20	.19	------	------
Total	12.33	19.36	10.77	14.23	21.61	10.83	10.99	15.33	13.07	2.22	1.59

DRY MATTER (PERCENTAGE OF THE WEIGHT OF FRESH ROOTS)

1	20.7	21.4	20.3	17.9	17.9	19.6	18.2	24.8	18.0	31.88	16.73
2	25.4	25.6	22.4	26.3	24.2	21.9	22.9	25.1	22.7	23.72	18.65
3	26.1	27.2	21.8	23.3	21.5	19.4	20.4	23.2	20.1	22.75	22.68
4	28.0	25.7	20.3	23.2	19.7	17.5	18.4	20.9	16.9	18.19	18.61
5	28.3	28.0	19.5	22.4	19.6	15.9	16.8	17.0	16.0	16.17	17.18
6	29.3	30.3	20.1	24.7	20.7	15.7	14.8	16.8	15.2	13.10	16.93
7	23.9	28.4	22.1	21.9	21.7	16.9	14.1	16.1	15.4	13.90	17.78
8	------	29.0	22.2	23.8	20.5	------	------	14.7	14.9	------	------

TOTAL AVAILABLE CARBOHYDRATES (PERCENTAGE OF DRY WEIGHT OF ROOTS)

1	15.96	12.81	19.28	10.77	10.85	8.99	11.96	8.37	7.59	10.18	4.11
2	20.96	18.74	16.83	15.69	9.82	9.85	11.01	11.72	6.78	4.21	3.54
3	23.62	30.92	19.04	20.45	14.77	10.73	8.03	14.12	7.74	4.25	3.46
4	27.06	38.49	24.12	25.08	20.06	13.24	8.23	15.55	7.80	6.01	3.33
5	41.90	37.91	35.46	35.01	25.14	22.05	11.64	15.22	8.59	2.80	2.81
6	50.78	51.35	46.71	38.10	33.79	26.84	12.43	13.05	9.48	2.87	3.94
7	51.95	53.50	42.72	39.30	36.16	34.60	16.35	14.96	9.26	4.20	2 36
8	------	------	42.45	42.57	31.16	------	------	15.26	8.21	------	------

TOTAL AVAILABLE CARBOHYDRATES (GRAMS PER CUBIC FOOT OF SOIL)

1	0.610	0.507	0.495	0.378	0.694	0.121	0.251	0.330	0.104	0.003	0.001
2	.590	1.056	.348	.361	.439	.246	.302	.516	.258	.022	.015
3	.468	1.073	.318	.587	.635	.247	.153	.394	.192	.027	.016
4	.271	.822	.413	.552	.481	.213	.115	.353	.160	.026	.009
5	.704	.648	.418	.497	.447	.198	.141	.137	.165	.007	.007
6	.330	.349	.374	.335	.419	.158	.104	.065	.095	.006	.004
7	.109	.417	.179	.306	.228	.066	.041	.049	.035	.004	.002
8	------	------	.149	.106	.097	------	------	.031	.016	------	------
Total	3.082	4.872	2.694	3.122	3.440	1.249	1.107	1.875	1.025	.095	.054

first year of sampling. On the other hand, there was a distinct decrease in percentage of available carbohydrates on all plots. After the first year of cultivation both roots and percentage of available carbohydrates decreased.

Data from plots 42 and 142 indicate a substantial loss between the last sampling dates in the fall and the first in the spring, which could have been due to growth in the spring before the first samples were

taken, or to variation in root quantity and in reserves from one place to another in the field.

It frequently was observed in this study that the roots died from the top down. This is illustrated in figure 3 which shows a bindweed root that had been killed by cultivation to a depth of about 30 inches and another killed to a depth of 59 inches, each of which produced rhizomes that eventually reached the surface of the soil and produced leaves.

TABLE 3.—*Quantity of roots and dry matter, and total available carbohydrates in the roots of European bindweed in plot 142*

QUANTITY OF ROOTS (GRAMS PER CUBIC FOOT OF SOIL)

Depth (feet)	1939				1940			
	May	June	Aug.	Oct.	May	June	Aug.	Oct.
1	2.16	2.16	1.41	2.30	1.74	1.34	0.26	0.28
2	2.04	2.30	1.56	2.31	1.78	2.30	.93	.94
3	1.70	1.87	1.06	1.72	1.08	1.98	.80	1.11
4	1.36	1.55	.98	1.34	.76	1.20	.70	.66
5	1.01	1.55	.68	.90	.44	.75	.48	.56
6	.46	.43	.31	.46	.22	.62	.27	.47
Total	8.73	9.86	6.00	9.03	6.02	8.19	3.44	4.02

DRY MATTER (PERCENTAGE OF THE WEIGHT OF FRESH ROOTS)

1	19.9	17.3	15.5	17.9	18.2	16.9	20.04	11.76
2	24.9	20.5	19.7	22.2	21.6	22.1	21.81	20.44
3	24.7	23.0	18.2	20.7	21.0	20.5	19.91	20.12
4	26.4	20.5	17.3	18.1	22.9	18.2	17.73	19.68
5	17.3	20.6	18.5	16.4	20.3	17.2	16.84	16.36
6	20.5	22.3	18.7	18.7	18.2	15.3	15.69	14.93

TOTAL AVAILABLE CARBOHYDRATES (PERCENTAGE OF DRY WEIGHT OF ROOTS)

1	0.285	0.157	0.098	0.212	0.162	0.074	0.010	0.019
2	.273	.125	.068	.109	.195	.090	.030	.035
3	.255	.108	.053	.074	.134	.101	.037	.033
4	.292	.133	.056	.077	.088	.072	.034	.025
5	.283	.262	.068	.052	.040	.047	.028	.025
6	.176	.096	.051	.043	.024	.068	.015	.015
Total	1.564	.881	.394	.567	.643	.452	.154	.152

TOTAL AVAILABLE CARBOHYDRATES (GRAMS PER CUBIC FOOT OF SOIL)

1	13.20	7.28	6.92	9.22	9.33	5.50	3.39	6.86
2	13.39	5.43	4.39	4.74	10.94	4.36	3.25	3.74
3	15.01	5.75	4.95	4.32	12.42	5.12	4.61	2.98
4	21.49	8.60	5.76	5.79	11.59	6.00	4.95	3.81
5	27.97	16.90	10.04	5.79	9.19	6.25	5.77	4.55
6	38.20	22.28	16.59	9.35	10.99	10.92	5.44	3.25

Especially after a severe winter but not limited thereto, the roots may be dead to a depth of a foot or more. The depth to which it was necessary to dig in order to find live roots became greater as the cultivation period was extended. A plausible explanation for this would be the inability of the plant to translocate available carbohydrates upwards rapidly enough to keep pace with growth. If this is the correct explanation, the methods of the present study were not sufficiently refined to show it. Another possibility is that roots tend to die when

the concentration of available carbohydrates falls below a certain minimum. In these studies the concentration in the upper part of the root was low to begin with as compared with that at greater depths and may have reached a level that was not sufficient to maintain life in the roots. Whether such was the case, however, cannot be determined from the data.

It appears to be significant, in relation to root-sampling studies, that the depletion in carbohydrates with cultivation seemed to take place as rapidly or even more rapidly at the lower levels than near the surface. The differences in rates of depletion are not great, however, and sampling to a depth of 2 feet only in these plots would have provided a substantially correct picture of the comparative reserves. The rate of depletion may be a function of the relative concentration at different levels, being more rapid at those soil horizons and in those cases where the concentration is greatest.

As mentioned above, the percentage of carbohydrates appears to reach a minimum level several weeks or months before all plants are dead and except for slight differences due to translocation from lower to upper levels seems to remain constant to the end within the limits of sampling error. This level was about 7 or 8 percent in plot 42, about 3 percent in plot 72, and 3 or 4 percent in plot 142. Probably much of this represents a systematic error in analysis or, more accurately, an error in designating as "available" the reducing sugar and "starch equivalent." Actually, as pointed out by Bakke, Gaessler, and Loomis (*2*), and recognized at the outset of this study, a small proportion of the material separated by the methods employed is not available to the plant. The error, however, is not regarded as important so far as the purpose of the present study is concerned. Why there should have been a larger quantity of this unused material in the roots of plot 42 than in the others is not known.

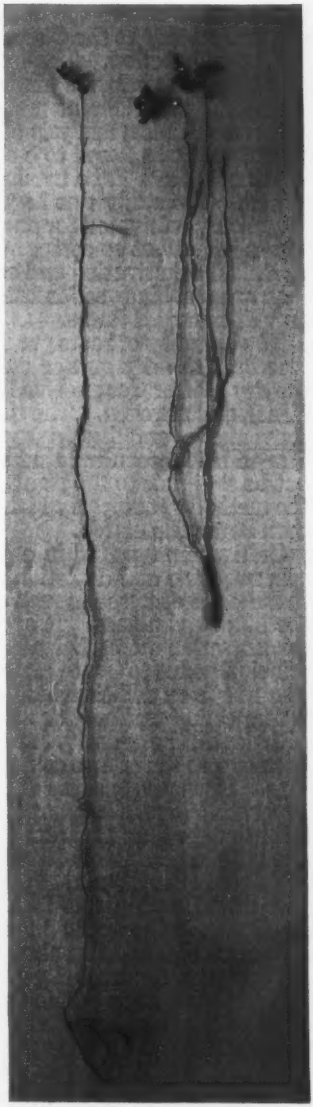

FIGURE 3.—Two European bindweed roots killed by cultivation to depths of 30 and 59 inches with rhizomes reaching the soil surface and above.

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SUMMARY

The effect of cultivation on root quantity and on root reserves in the European bindweed (*Convolvulus arvensis* L.)at various depths in the soil was studied at the Cherokee State Hospital, Cherokee, Iowa, during a 3-year period. These studies were made on land that probably had been infested with bindweed for 20 years or more. The studies included three different plots. On two of them sufficient bindweed roots for chemical analysis (about 4 gm. of dry material from 25 cubic feet of soil) was found to a depth of 8 feet and on the third plot to a depth of 6 feet. The maximum penetration of roots was not determined. The study was begun on one of the plots after it had been intensively cultivated for one season, which probably reduced root reserves somewhat as compared with normally cultivated and cropped land. The cultivation and cropping on the others was more intensive than on most bindweed-infested fields though probably no more so than on well-managed farms.

The quantity of roots per unit volume of soil for each foot level was recorded, the dry-matter content of the roots was determined, and the dry matter was analyzed for reducing sugars, starch (starch equivalent), and nitrogen. The sugars and starch were added together for convenience and herein reported as total available carbohydrates, though it is recognized that a part of the material so designated may not be available to the plant. This is probably due in part at least to the method of analysis which is known to include some material other than sugar and starch.

Cultivation resulted in a gradual and continuous reduction in the concentration and depletion of the quantity of total available carbohydrates, a decrease in root quantity, and eventually in the death of the bindweed plants. It appeared that cultivation first affected the concentration of carbohydrates, the depletion in root weight being principally due to loss of carbohydrates. Later the roots began to die, resulting in an additional reduction of the weight of living roots.

The concentration of available carbohydrates markedly increased with depth, the quantity expressed as a percentage of dry matter being from two to four times as much in the lower as in the upper soil horizons. However, because of the much greater quantity of roots in the upper soil layers, the quantity of carbohydrates in these layers was also much greater than at the lower depths.

At the beginning of the sampling periods approximately 25 percent of the root quantity and 18 percent of the total available carbohydrates were found in the upper foot of soil and about 49 and 38 percent, respectively, in the upper 2 feet of soil.

General observations showed that the roots died first in the upper soil horizons, the least depth at which living roots could be found increasing with time. This suggests that the conversion of starch to sugar or the translocation of sugar from the lower levels was not sufficiently rapid to keep the roots alive. However, no consistent differences in the rate of depletion of starch or sugars at different depths such as would account for this could be demonstrated. In some cases the rate of depletion of carbohydrates was more rapid at the lower soil levels than near the surface, but in other cases the reverse seemed to be true.

An accurate picture of root reserves in European bindweed cannot be had without considering both the concentration of carbohydrates and root quantities in all soil horizons in which there are appreciable root quantities. Since, however, the greater proportion of roots and available carbohydrates are found in the upper layers of soil, it appears that a close approximation can be secured by sampling the upper 2 feet only.

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ADDITIONAL STRAINS OF THE SUGAR-BEET CURLY TOP VIRUS [1]

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INTRODUCTION

Four strains of the sugar-beet curly top virus were reported in 1938 [2] as having been clearly differentiated. At that time it was evident that there were other strains; the present paper reports the results of further work in the isolation and differentiation of these.

The term "selection," as applied to viruses in this paper, indicates that a virus was selected for further study, because of some peculiarity in host reaction or because of an unusual host or a new location, upon the assumption that such a selected virus might be found to be an undescribed strain of curly top. Therefore the term "selection" is considered more appropriate than either "isolation" or "culture."

When new selections of any virus are being used, there is always some uncertainty as to whether a single strain or a mixture of two or more strains may be present. Accordingly great care has been taken to avoid describing such strain combinations as new strains.

SOURCES OF VIRUS STRAINS

Six additional strains (Nos. 5, 6, 7, 8, 9, and 10) have been segregated from a large group of curly top virus selections. Strains 5, 6, and 10 were obtained in 1937 from plants of tree tobacco (*Nicotiana glauca* Graham) which had grown in natural breeding areas of the beet leafhopper (*Eutettix tenellus* (Bak.)) near Tracy, Calif. Strain 7 was obtained in 1937 from diseased spinach plants (*Spinacia oleracea* L.) growing in a commercial field west of Hemet, Calif. Strain 8 came under test in 1936 as a result of inoculations by J. M. Wallace [3] on Red Mexican bean (*Phaseolus vulgaris* L.) with a colony of viruliferous leafhoppers carrying a mixture of unknown virus strains. Strain 9 was obtained earlier from a similar colony by inoculations on a resistant beet (*Beta vulgaris* L.) but was first recognized as a distinct selection at the same time as strain 8 and as a result of the same group of inoculations.

[1] Received for publication March 3, 1943.

[2] GIDDINGS, N. J. STUDIES OF SELECTED STRAINS OF CURLY TOP VIRUS. Jour. Agr. Res. 56: 883-894, illus. 1938.

[3] Unpublished data from the records of J. M. Wallace, formerly of the Division of Sugar Plant Investigations.

DIFFERENTIAL HOSTS

Plants tested for possible value as differential hosts include 47 species and varieties, besides a number of varieties of cultivated sugar beet (*Beta vulgaris*). These plants may be divided into two groups, one containing those that became infected by one or more strains of the curly top virus and the other those in which none of the virus strains produced infection.

SPECIES OR VARIETIES IN WHICH INFECTION WAS OBTAINED

CHENOPODIACEAE:

Beta macrocarpa Guss.
Beta patellaris Moq.
Beta trigyna Waldst. and Kit.
Beta vulgaris
Chenopodium murale L. (nettleleaf goosefoot)
Spinacia oleracea (spinach, var. Improved Prickly Winter)

COMPOSITAE:

Lactuca sativa L. (lettuce, var. Los Angeles Head)

CRUCIFERAE:

Erysimum repandum L.
Lepidium lasiocarpum Nutt.
Lepidium nitidum Nutt. (common peppergrass)
Malcolmia maritima (L.) R. Br. (Virginian stock)

GERANIACEAE:

Erodium cicutarium (L.) L'Hér. (redstem filaree)

HYDROPHYLLACEAE:

Nemophila maculata Lindl. (spotted nemophila)

LEGUMINOSAE:

Lathyrus odoratus L. (sweet pea)
Medicago hispida Gaertn. (bur-clover)
Melilotus indica All. (yellow sweetclover)
Phaseolus vulgaris (bean, var. Bountiful)
Phaseolus vulgaris (bean, var. Great Northern)
Phaseolus vulgaris (bean, var. Red Mexican)
Trifolium hybridum L. (alsike clover)
Trifolium incarnatum L. (crimson clover)
Trifolium repens L. (white clover)

PAPAVERACEAE:

Eschscholtzia californica Cham. (California-poppy)

PLANTAGINACEAE:

Plantago erecta Morris.
Plantago major L. (common plantain)

PRIMULACEAE:

Samolus parviflorus Raf. (water pimpernel)

SOLANACEAE:

Datura stramonium L. (jimsonweed)
Lycopersicon esculentum Mill. (tomato, var. Improved Stone)
Lycopersicon esculentum (tomato, var. Earliana)
Lycopersicon esculentum (tomato, var. John Baer)
Nicotiana glauca (tree tobacco)
Nicotiana glutinosa L.
Nicotiana tabacum L. (tobacco, var. Turkish)
Solanum tuberosum L. (potato, seedlings)

VIOLACEAE:

Viola tricolor L. (pansy)

Some hosts in which infection was obtained were susceptible to all curly top strains tested on them, whereas others were infected by very few. The plants

thus far found most useful in differentiating curly top virus strains are the susceptible sugar-beet selection S. L.[4] 842, the highly resistant sugar-beet selection 68,[5] Turkish tobacco, the highly resistant Red Mexican bean, and the very susceptible Bountiful bean.

SPECIES OR VARIETIES IN WHICH INFECTION WAS NOT OBTAINED

CRUCIFERAE:

Brassica adpressa (Moench) Boiss.
Malcolmia africana (L.) R. Br.

LEGUMINOSAE:

Medicago sativa L. (alfalfa)
Trifolium pratense L. (clover, var. Mammoth)
Trifolium pratense (clover, var. Red)
Trifolium repens L. (white clover, var. Ladino)

MALVACEAE:

Gossypium hirsutum L. (cotton)
Hibiscus esculentus L. (okra, var. Early Greenpod)

MYRTACEAE:

Feijoa sellowiana Berg (pineapple guava)

PUNICACEAE:

Punica granatum L. (pomegranate, seedlings)

ROSACEAE:

Malus sp. (apple, var. Winesap, seedlings)
Rosa chinensis Jacq. (rose, var. Ragged Robin, seedlings)

METHODS

The general methods used in studies to differentiate these strains were the same as those described in the report by the author on the first four strains,[6] except for important changes in the method of inoculating beans. In 1937, J. M. Wallace [7] inoculated Red Mexican bean with curly top virus and secured good evidence of infection and injury. The author had reported [6] that no case of infection was obtained by inoculation of Red Mexican bean with any curly top strains listed at that time; so notes and methods were compared. All the author's inoculations had been made before the second leaf opened out, and the viruliferous leafhoppers were caged [8] on the first or second (unfolding) leaf. Wallace had made inoculations before any leaf opened out by placing a large celluloid cage over the entire young plant. The leafhoppers were thus able to feed upon the cotyledons very soon after they appeared above ground. Wallace discovered that certain strains of the curly top virus would produce infection of Red Mexican bean if the leafhoppers fed upon the cotyledons, but that the same strains would not produce infection if the leafhoppers were allowed to feed only on young leaves of the plant. After learning this the writer made all bean inoculations so that the leafhoppers could feed on the young cotyledons. Wallace's inoculations on Red Mexican bean showed that the two curly top strains

[4] Designation of seed lot at the Salt Lake City Station, Salt Lake City, Utah, of the Division of Sugar Plant Investigations.
[5] Formerly designated as strain 1167.
[6] See footnote 2.
[7] See footnote 3.
[8] GIDDINGS, N. J. A SMALL CAGE FOR INSECT VECTORS USED IN PLANT INOCULATIONS. Phytopathology 29: 649-650, illus. 1939.

now designated as 8 and 9 produced distinctly more severe symptoms on that host than any of the other virus strains available at that time.

All evidence here reported in relation to strain differentiation is the cumulative result of repeated inoculations with leafhoppers that had fed for some time on a plant infected with a given virus strain and is taken from records that include percentage of plants infected, relative severity of symptoms, and plant mortality. The severity rating is

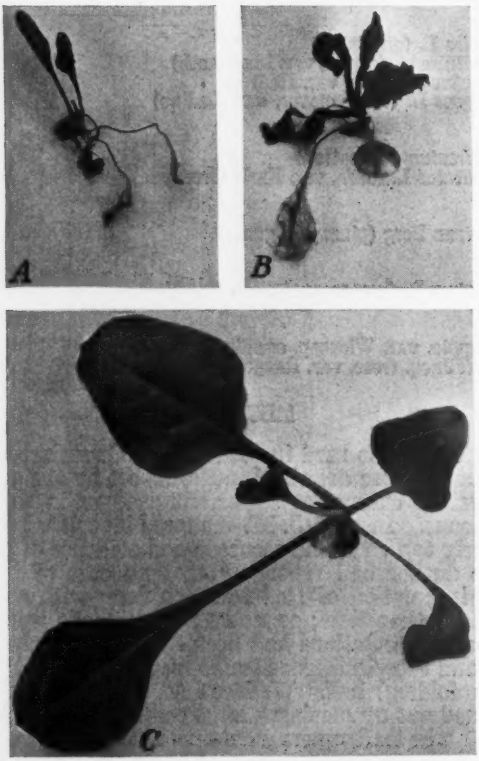

FIGURE 1.—Susceptible sugar-beet plants 40 days after infection by curly top virus, strain 6 (*A*) (*+++ in table 2) and strain 3 (*B*) (+++ in table 2); *C*, healthy check plant.

based on a scale of five grades, in which 1 indicates slight symptoms and 5 indicates very severe symptoms. Earlier published descriptions and illustrations of these grades of severity by the author [9] require some revision in view of newly discovered symptoms. The earlier and the revised description for each grade are given in table 1. The two types of grade 5 symptoms are shown in figure 1.

[9] See footnote 2.

TABLE 1.—*Comparison of original and revised descriptions of curly top severity grades*

Description	Curly top severity grade No. —				
	1	2	3	4	5
Original	Either slight vein clearing or occasional papillate growths on under side of foliage, or both.	Either slight curling of leaves with pronounced vein clearing or numerous papillae, or both.	Pronounced curling and some dwarfing.	Pronounced curling and increased dwarfing.	Extreme curling and dwarfing.
Revised	....do....	Generally as above. Newly discovered symptoms may cause confusion because of relatively little distortion even in plants in severity grades 3, 4, or 5, which are usually a darker green.	As above, or dwarfing without pronounced curling, but usually a darker green.	As above, or increased dwarfing without pronounced curling, but usually a darker green, with a rather high mortality rate.	As above, or extreme dwarfing without much curling, but usually a darker green, with a very high mortality rate.

EXPERIMENTAL RESULTS

EVIDENCE INDICATING THAT THE SELECTIONS ARE NEW STRAINS

Strains 5 and 6, when inoculated into young susceptible beets, induce characteristic symptoms quite different from those considered usual for curly top on that host. Infected small plants usually show little or no leaf distortion (fig. 1) and frequently have a deeper green color than is found in other diseased or healthy plants. There is extreme dwarfing of infected plants and a very high percentage of mortality among them. Plants infected in the three-leaf stage or later are not so likely to show the typical symptoms just described. Strain 5 has been most easily differentiated from strain 6 by the greater severity of symptoms it induces on Turkish tobacco (fig. 2). Strain 5 appears to be more virulent than strain 6 on *Nicotiana glutinosa*, whereas strain 6 seems more virulent than strain 5 when inoculated into Red Mexican bean, University of Idaho No. 15 selection of Great Northern bean, and the highly resistant sugar-beet variety 68.

Strain 7 is distinctly the least virulent of any thus far selected, and it is the only strain that has never given at least an occasional symptom on the resistant beet variety 68. It does not infect Turkish tobacco and only rarely infects *Nicotiana glutinosa*, producing very mild symptoms that are scarcely discernible. The curly top susceptible bean variety Bountiful, inoculated through the cotyledons, was rather quickly killed by most of the virus strains thus far tested; but plants of this variety infected by strain 7 were rarely killed and most of them made further growth (fig. 3) after pronounced curly top symptoms had been in evidence for some time. Infected spinach plants are usually severely stunted or killed by infection with any of the curly top strains except strain 7. It induces appreciable injury but much less than that caused by any of the other strains.

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Strains 8 and 9 are very similar in virulence on most host plants tested, but strain 9 infects a higher percentage of plants of the resistant beet 68 and produces distinctly more severe symptoms than strain 8 upon the Red Mexican bean (fig. 4).

Strain 10, when inoculated into the young susceptible sugar-beet plants, produces symptoms quite similar to those induced by strains 5 and 6; but the severity is much less in the case of strain 10, and very few of the infected plants die. Its general host range is the same as that of strains 5 and 6, but the symptoms are much less severe

FIGURE 2.—Turkish tobacco plants 39 days after infection by curly top virus, strain 5 (*A*) and strain 6 (*B*); *C*, healthy check plant of same age.

on each of the hosts thus far tested, with the possible exception of tomato.

Table 2 indicates the differential host reactions by which these strains and the previously described strains 1, 2, 3, and 4 may be distinguished. For presenting evidence on infection, the symbol + indicates that a high percentage of plants were infected, − indicates that a small percentage of plants were infected, and 0 that there were no plants showing symptoms. The severity of symptoms is classed as extremely slight (− −), slight (−), moderately severe (+), severe (+ +), and very severe (+ + +). Although these symbols are less precise than the means of a series of numerical grades, they are convenient classifications into which the actual readings are easily translated and constitute a much more usable method of expressing the differential reactions obtained with these virus strains.

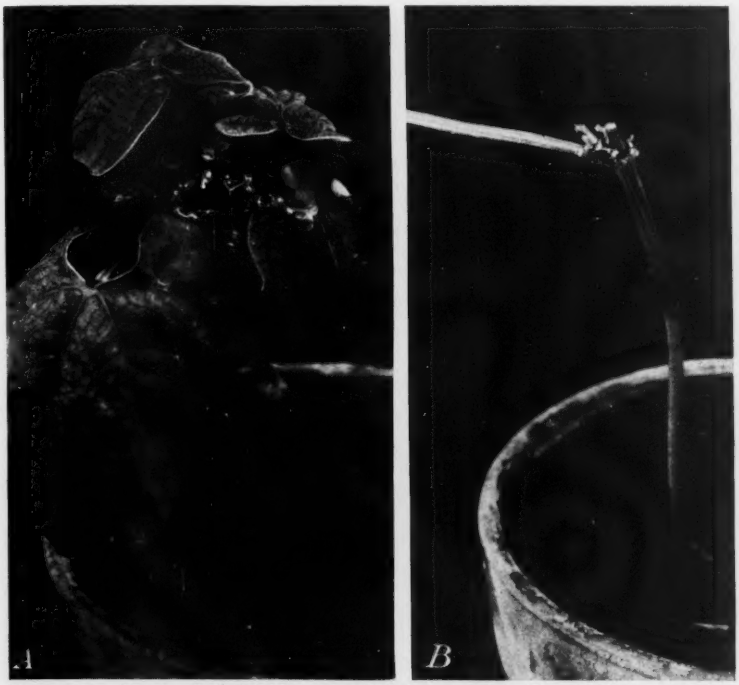

FIGURE 3.—Plants of the highly susceptible bean variety Bountiful 56 days after inoculation in cotyledon with curly top virus: *A*, Plant infected by virus strain 7 has survived and developed some new growth although it still shows very severe symptoms; *B*, plant infected by virus strain 2, which is less virulent than any of the other strains tested on Bountiful bean except strain 7.

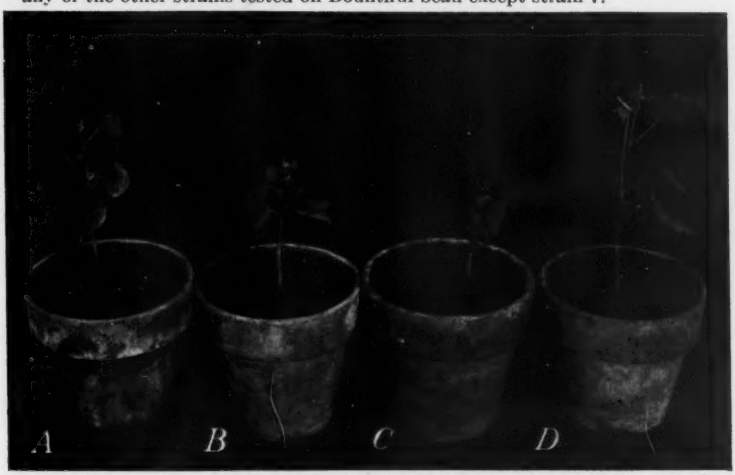

FIGURE 4.—Plants of the highly resistant bean variety Red Mexican 20 days after inoculation in cotyledon with different strains of curly top virus: *A*, Strain 3; *B*, strain 8; *C*, strain 9; *D*, uninoculated check.

The data upon which table 2 is based have been evaluated statistically, and all differences are highly significant.

TABLE 2.—*Key for differentiating 10 strains of curly top virus by means of a susceptible variety (S. L. 842) and a highly resistant variety (68) of sugar beets, Turkish tobacco, and Red Mexican bean*

Strain	Susceptible sugar beet		Resistant sugar beet		Turkish tobacco		Red Mexican bean	
	Infection [1]	Severity of symptoms [2]	Infection [1]	Severity of symptoms [2]	Infection [1]	Severity of symptoms [2]	Infection [1]	Severity of symptoms [2]
1	+	++	+	+	+	++	−	−
2	+	−	+	−	0	0	−	−−
3	+	+++	−	−	+	++	+	−
4	+	−	−	−	0	0	−	−−
5	+	*+++	−	−	+	+++	−	−−
6	+	*+++	−	−	+	++	+	−
7	+	−−	0	0	0	0	0	0
8	+	++	−	−	+	++	+	+
9	+	+++	+	−	+	++	+	++
10	+	*+	−	−	+	−	−	−−

[1] 0=no plants infected; −=low percentage of plants infected; +=high percentage of plants infected.
[2] −−=extremely mild symptoms; −=mild or slight symptoms; +=moderately severe symptoms; ++=severe symptoms; +++=extremely severe symptoms; *=not much distortion of infected plants by strains 5, 6, or 10 but high mortality in case of strains 5 and 6.

STABILITY OF THE VIRUS STRAINS

Each of the curly top virus strains mentioned has been carried for several years, has been tested occasionally, and has shown a high degree of stability. Despite the stability of all these strains, any one of them may induce symptoms that show distinct differences in severity. For example, 20 susceptible plants infected by strain 7 and held under the same environmental conditions may quite commonly show 3 or 4 plants with symptoms graded as 2.0 on the scale of severity, a few graded as 1.5, and the remainder as 1.0. Such variation may be due to inherent differences between the individual plants or to environmental factors. In the case of strains 2, 4, and 7, there have been repeated selections of plants that showed a severity grade higher than the average for the strain under consideration, and in no case have any of these yielded a virus more virulent than the original selection of that strain. Similarly, there have been selections from the less severely diseased plants infected by strains 1 and 3, and in no case was there good evidence that a less virulent strain was obtained by this method.

A further effort was made to increase the virulence of strains 2, 4, and 7 by inoculation of very young susceptible plants and successive transfers from such plants just as soon as the first symptoms appeared. There was no evidence of increased virulence in any case.

DISCUSSION AND SUMMARY

The 10 virus strains reported in this paper are considered to be a part of a much larger group having the same vector, the same general host range, and similar symptoms. They may, therefore, be considered under one group designation as curly top virus, *Ruga verrucosans* Carsner and Bennett.[10]

[10] CARSNER, E., and BENNETT, C. W. NAME AND CLASSIFICATION OF THE CURLY-TOP VIRUS. Science 98: 385–386. 1943.

Each of the strains appears to be an entity which may be recovered from mixtures of strains and seems very stable. Because of the characteristics that they have in common, and as a matter of convenience, it seems best to designate these selections by strain number rather than as varieties of the species *Ruga verrucosans*.

A highly susceptible beet or other plant is essential for recognizing the presence of the virus. Any of the curly top resistant beets now available would readily serve to test for the presence of strain 1 or 2 but might not give any clear evidence concerning such highly virulent strains as 3, 5, or 6.

Host plants that effectively differentiate the various strains are necessary for progress in separating and classifying them, and more such plants are being sought. The 10 strains already differentiated present a picture of the plant reactions that have thus far been recorded.

The present paper describes a previously unidentified reaction, which consists of extreme dwarfing of young, susceptible sugar-beet plants without the pronounced distortion hitherto considered the chief symptom of severe injury. A high percentage of mortality among infected sugar-beet plants is usually associated with this newly described symptom.

The mere fact that a virus strain produces severe symptoms on one host is no definite indication as to the reaction that may occur with other hosts. Arrangement of the 10 described strains according to an ascending scale of amount of injury induced in the susceptible sugar beet would be approximately 7, 2, 4, 10, 1, 8, 3, 9, 5, 6. A similar arrangement according to injury in the resistant beet variety 68 would be 7, 10, 3, 8, 5, 6, 4, 2, 9, 1. Strain 7 holds the same relative position for each of these 2 beets, but strain 2 moves from second place on the susceptible beet to eighth place on the resistant beet. Strains 4 and 1 also show a much higher relative severity rating on the resistant beet than on the susceptible. On the other hand, strains 3, 5, and 6, which produced very severe symptoms on the susceptible beet, were relatively low in their rating on the resistant beet. Strains 2 and 4, which rate relatively high on the resistant beet, and strain 7, which is low on both beets, form a group that has never produced infection on Turkish tobacco. However, strain 10 produces mild symptoms, strain 6 rather severe symptoms, and strains 1, 3, 5, 8, and 9 produce very severe symptoms on that host. On the Red Mexican bean, strains 8 and 9 were the only ones that induced comparatively severe injury and strain 9 was clearly more virulent than strain 8.

Adequate knowledge of various curly top strains has an important bearing upon the disease-resistance breeding program for other host plants as well as for sugar beets.

BIOLOGY OF ALLOTROPA BURRELLI, A GREGARIOUS PARASITE OF PSEUDOCOCCUS COMSTOCKI[1]

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INTRODUCTION

In the fall of 1939 a shipment of a gregarious platygasterid, *Allotropa burrelli* Mues.,[2] was received at Moorestown, N. J., from the Yokohama, Japan, station of the Division of Foreign Parasite Introduction, Bureau of Entomology and Plant Quarantine. The primary purpose of the importation was to furnish breeding stock for propagating parasites of this species for release against the Comstock mealybug (*Pseudococcus comstocki* (Kuw.)), which has recently become a serious pest of apple in sections of Virginia, West Virginia, and Ohio.

During the course of the propagation work at Moorestown, from January 1940 to January 1941, the author had an excellent opportunity to make observations on the biology of this species, a member of a very interesting and little-known group. The genus *Allotropa* contains only three other described North American species, *A. convexifrons* Mues. from the Comstock mealybug,[2] *A. utilis* Mues. from *Phenacoccus aceris* (Sign.),[3] and *A. ashmeadi* Mues. from an undetermined mealybug.[3]

A special attempt was made to determine whether polyembryony occurs in this platygasterid. All drawings and observations were made from fresh material dissected in normal salt solution, without special stains or sectioning. Consequently, the precleavage development of the egg was not followed.

THE ADULT

The adult is shining black and approximately 0.8 mm. long. The sexes can be most readily distinguished by differences in the antennae; the male has long, hirsute, moniliform antennae, whereas the female has shorter, sparsely pubescent, and distinctly clavate antennae. The insects are fairly active at room temperatures, although they rarely attempt flight.

At 80° F. adults live on an average but 3 to 5 days. Even at lower temperatures they seldom live more than 10 days.

Males generally begin emerging first and remain on or near the mummies[4] to assist and mate with the females. The eggs are deposited at random in the host's body cavity, and oviposition begins immediately if hosts are present. After a brief examination, the

[1] Received for publication November 24, 1942.

[2] MUESEBECK, C. F. W. TWO NEW SPECIES OF ALLOTROPA (PLATYGASTERIDAE, SERPHOIDEA) PARASITIC ON THE COMSTOCK MEALYBUG. Brooklyn Ent. Soc. Bul. 37, pp. 170–173, illus. 1942.

[3] MUESEBECK, C. F. W. A NEW MEALYBUG PARASITE (HYMENOPTERA: SCELIONIDAE). Canad. Ent. 71: 158–160, illus. 1939.

[4] In this paper the term "mummy" refers to the inflated host derm containing parasite larvae or pupae within the typical hardened cells.

female turns about, thrusts her ovipositor backward into the mealybug, and then remains motionless for 5 to 10 seconds, often being dragged along by the host until oviposition is completed. Under favorable conditions the female parasitizes several hosts in quick succession, thereby depositing most of her eggs in a short time—an obvious necessity in view of her short life span.

Mealybug nymphs of all stages are attacked, although preference appears to be shown for those at least half grown. Small hosts are generally able to become nearly mature before being mummified, although the developmental period is somewhat lengthened.

THE EGG AND THE PARASITE BODY

The ovarian eggs are exceedingly small and numerous; they average 0.056 by 0.010 mm. in size (fig. 1, *A*). From 332 to 784 eggs per female, with an average of 565, have been disclosed by dissection. The ovarian eggs are nearly all mature when the female emerges, although the ovarioles may produce additional ova as deposition proceeds.

After oviposition at 75°–80° F., the deposited egg remains outwardly unchanged for approximately 24 hours. Within the next 12 hours, however, it commences to shorten and becomes more spherical in outline, until after 36 to 48 hours it is perfectly round and measures about 0.025 mm. in diameter (fig. 1, *B*). At this stage the central body is well defined, either round or oval, and distinctly granular in appearance. It is obviously identical with the "masse vitelloide" of the monembryonic platygasterid *Synopeas rhanis* Walk.,[5] although the embryonic nuclei are not yet apparent. It is surrounded by a circular clear area which is variable in extent (fig. 1, *B* and *C*), and outside of that is a ring of gray, semiopaque material which is probably the trophamnion. Eggs in this stage can be distinguished from the fat cells and other of the host contents only by their typical organization. Owing to the quantity of host tissue encountered, it was impossible to identify eggs prior to this stage after they had become spherical in outline.

Intermixed with these eggs are numerous others that have attained the morula stage (fig. 1, *D*), together with many in the premorula stage (fig. 1, *B*). All stages are present approximately 2 days after oviposition, owing to individual differences in rate of development. Since the egg begins to increase in size during the morula stage, it is henceforth known as the parasite body. It now averages 0.036 mm. in diameter, and generally contains five or six embryonic nuclei around the central body. It will be noted that the entire embryonic region is slightly off center. The paranucleus was not observed in the temporary unstained mounts used, although on several occasions faint partial outlines of the paranucleus were noted.

After about 2½ days the parasite body enters the early blastula stage (fig. 1, *E*) and measures approximately 0.045 mm. The paranucleus is now a distinct round to oval-crescentic body located in the wider portion of the trophamnion. The embryonic nuclei vary in number, and each consists of an inner and an outer layer; this double-bodied effect is also frequently noticeable among late morulae.

[5] MARCHAL, P. RECHERCHES SUR LA BIOLOGIE ET LE DÉVELOPPEMENT DES HYMENOPTÈRES PARASITES LES PLATYGASTERS. Arch. de Zool. Expt. et Gén. (IV) 4: 485–640, illus. 1906.

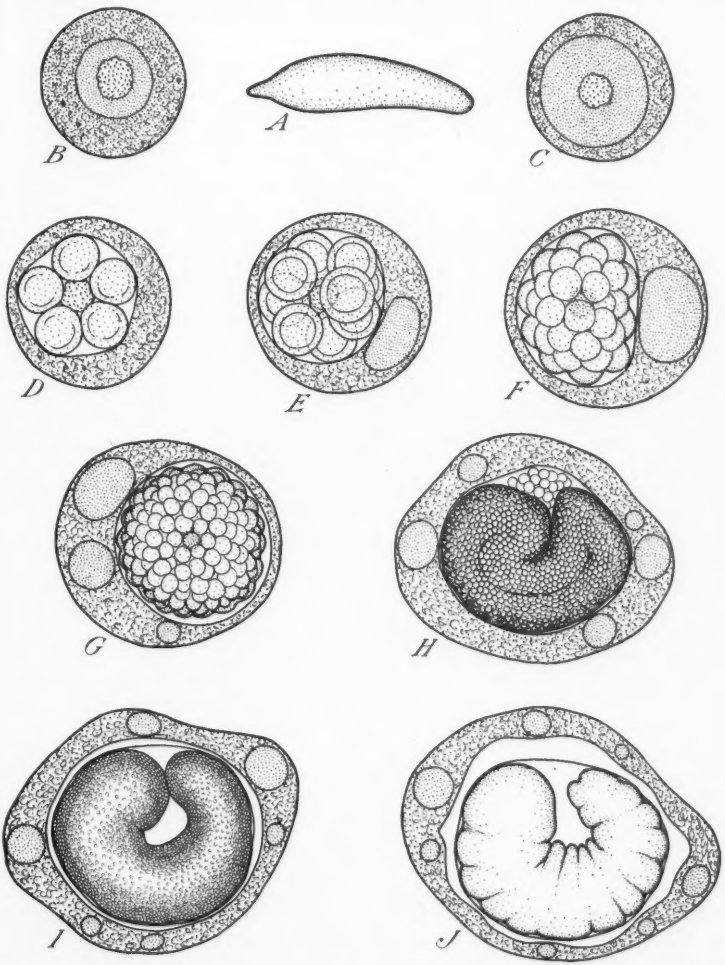

FIGURE 1.—Embryonic development of *Allotropa burrelli: A*, Ovarian egg; *B* and *C*, premorulae before appearance of embryonic nuclei; *D*, typical morula; *E*, early blastula, showing double-bodied embryonic nuclei and paranucleus; *F*, later blastula; *G*, late blastula with three paranuclear masses; *H*, gastrula, showing proliferated cells; *I*, later embryo undergoing organ and tissue formation; *J*, advanced embryo within delicate membrane.

About 3 days from oviposition the typical hollow sphere or blastula becomes apparent, generally with a single, large paranucleus in the periphery (fig. 1, *F*). The parasite body now averages 0.060 mm. in

diameter, and is readily distinguished from the surrounding host bodies.

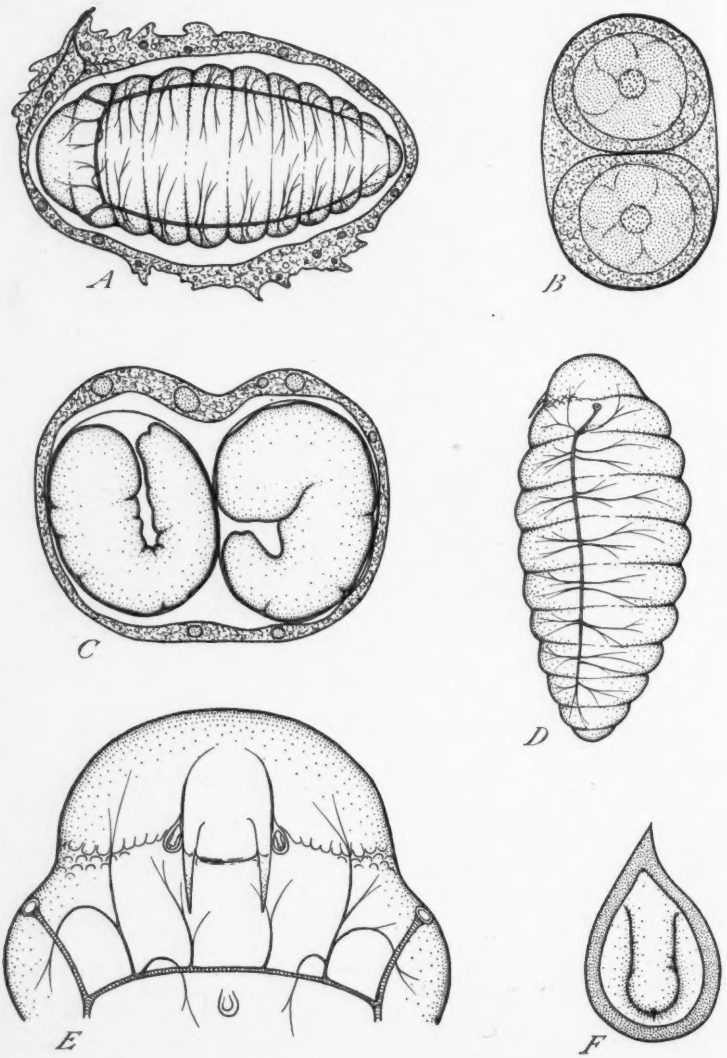

FIGURE 2.—Evidences of polyembryony and the larval morphology of *Allotropa burrelli*: *A*, Larva ready to emerge from degenerate trophamnion; *B*, twin early morulae within membrane, probably of host origin; *C*, twin embryos within a single trophamnion—definite evidence of polyembryony; *D*, mature larva; *E*, oral structures of larva; *F*, enlarged maxillary palpus.

During the fourth day the blastula attains its maximum development, with countless small cells surrounding the still faintly discernible

central body (fig. 1, *G*). The parasite body has become oval and slightly irregular in outline, averaging 0.090 mm. in greatest diameter, and contains from two to five paranuclear masses of various sizes.

In the late blastula and subsequent stages it becomes increasingly difficult to dissect out the entire parasite body, since the fragile trophamnion ruptures very easily.

Although the eggs are deposited free in the host fluids, during their development most, if not all, of them eventually become associated with the mealybug fat bodies. If the fat bodies are carefully teased out and pressed beneath a cover glass, the immature stages are seen adhering to the surface or partially embedded among the fat cells. As growth proceeds and nutriment is extracted by the various trophamnia, the fat bodies become depleted of their contents until, as the embryos approach maturity, all that remains is a tangled mass of host tissues and tracheae. The earliest stage found definitely associated with the fat body was the blastula.

The tendency toward cyst formation in the Platygasteridae by those species that develop in the coelomic fluids has been well demonstrated by Marchal [6] and Leiby and Hill.[7] Other species deposit their eggs in specific organs, such as the brain, ventral nerve cord, or intestine, where nourishment is readily obtained. Although *Allotropa burrelli* does not actually become encysted, its close relation to the host fat body undoubtedly serves the same purpose.

The gastrula stage (fig. 1, *H*) appears about the fifth day after oviposition. At first a slight indentation on one side is noted, which as it grows deeper gradually becomes filled with a loose mass of proliferated cells. These cells frequently appear late in the blastula stage, and may be involved in the formation of the delicate membrane which subsequently enfolds the embryo, although occasionally they are found within this membrane. The paranuclear masses vary in number and size, and the parasite body is typically irregular in outline, measuring about 0.180 mm. long.

By the following day germ-layer formation is generally complete and organogeny has commenced (fig. 1, *I*). The parasite body now averages about 0.31 mm., the trophamnion contains a variable number of paranuclear masses, and a delicate, tight-fitting membrane surrounds the embryo. At high magnifications the cells comprising the embryo can be barely distinguished.

The embryo 7 to 8 days old (fig. 1, *J*) is considerably advanced internally and has begun to exhibit body segmentation; it is now approximately full-sized, and the trophamnion has also attained its maximum development. Henceforth the paranuclear masses become smaller and less distinct and the trophamnion becomes thinner. At this stage the parasite body measures about 0.43 mm. in length, although it varies greatly in shape. Also at this time the host fat bodies have been practically exhausted of their contents, and are nearly obscured by the gelatinous parasite bodies which adhere to one another in shapeless masses. It is extremely difficult to tease them out entire.

Just before eclosion the embryo straightens out, rupturing the inner membrane and bringing the mandibles into contact with the trophamnion. These movements probably combine to effect hatching.

[6] See footnote 5, p. 160.

[7] LEIBY, R. W., and HILL, C. C. THE TWINNING AND MONEMBRYONIC DEVELOPMENT OF PLATYGASTER HIEMALIS, A PARASITE OF THE HESSIAN FLY. Jour. Agr. Res. 25: 337-350, illus. 1923.

The mature embryo, or unhatched larva, now averages 0.54 mm. in length (fig. 2, *A*), the parasite body having increased approximately 24 times in length. The tracheal system, however, rarely becomes filled with air, and thereby clearly distinguishable, until the trophamnion is broken.

According to all available evidence *Allotropa burreili* normally develops monembryologically. Three twin embryos were found out of the hundreds examined. Two of these pairs were identical early morulae (fig. 2, *B*), and were complete parasite bodies, each with a separate trophamniotic layer, tightly enclosed by a thin membrane possibly of host origin; they may represent twins from a single egg deposited by accident within a fat cell or other host body, or perhaps be the result of two eggs laid simultaneously therein. The third pair (fig. 2, *C*) was more advanced, and there is no doubt that embryonic fission had actually occurred, since both embryos were surrounded by a single trophamnion. That this phenomenon is either accidental or of rare occurrence is indicated by the finding of only one such individual.

It is possible that twinning sometimes takes place at a very early stage, as has been shown by Leiby and Hill [8] in *Platygaster hiemalis* Forbes. This could be demonstrated, however, only through a detailed embryological study involving special techniques of staining and sectioning. The present study has at least shown that monembryony is the rule (additional evidence is presented in a subsequent section), and that polyembryony, if it does occur, is of an extremely simple type.

THE LARVA

Eclosion takes place from 8 to 12 days after oviposition, the average being about 9½ days, at 75°–80° F. After the trophamnion is consumned, the various host tissues and organs are attacked, until within 1 to 3 days the larva has doubled in size, average measurements being 1.10 mm. by 0.50 mm. The host is not killed until its inhabitants are approximately half-grown, although it shows little activity after the parasite larvae emerge.

The larva of *Allotropa burrelli* (fig. 2, *D*) is peculiar in several respects. It possesses but one pair of spiracles, situated on the anterolateral regions of segment 1, and is without a posterior commissure. There are 10 well-defined segments in addition to the head, which bears ventrally an unusual and apparently heretofore undescribed series of oral structures (fig. 2, *E*). The slender, amber-tipped mandibles merge imperceptibly into the head, the tips measuring 0.063 mm. in length; they are capable of only very feeble movement. Between them lies the slitlike oral aperture, which is distinguishable in life by means of the undulating labrum, while immediately adjacent on each side are the maxillary palpi. Each palpus (fig. 2, *F*) extends slightly outward from the surface, and is tipped with a microscopic dark seta. A series of minute raised protuberances traverse the posterior margin of the head from the palpi to a point near the spiracles, while the salivary duct opens centrally in the region of the anterior commissure.

The ingested food materials are contained within a peritrophic membrane which forms a complete sac. If the larva is carefully

[8] See footnote 7, p. 163.

dissected, this sac floats free as a single unit; it is resilient and will bear considerable pressure beneath a cover glass before bursting.

That cannibalism occurs in *Allotropa burrelli* was proved by the finding of several young larvae impaled upon the mandibles of older individuals. Most of the body contents had been extracted, leaving the prey a shapeless mass distinguishable only by means of the tiny mandibles. Such examples were frequently encountered, particularly where development was uneven, the "stragglers" being eliminated in this manner. Cannibalism is apparently due to chance encounter rather than to aggressive action on the part of the parasite.

As the larvae become full-fed they lie closely packed together within the host derm. After 2 or 3 days each larva has formed about itself a parchmentlike cell or cocoon, which adheres to neighboring cocoons. The host is now rigidly distended into numerous small brownish "blisters." All attempts to locate the source of this cocoon-making material were unsuccessful; it may issue through the prominent salivary duct.

The brown, watery meconium is expelled approximately 2 days after cocoon formation, and wells up around the posterior half of the larva, where it soon hardens into an opaque layer. Larvae at this stage are identical in every respect, including oral structure, to those newly emerged or in the process of eclosion; therefore, only one instar occurs in *Allotropa burrelli*. Both *Platygaster hiemalis*[9] and *P. ornatus* Kieffer have also been shown to have but one instar.[9]

THE PUPA

Following a prepupal period of about 2 days, the pupal stage is begun. At first the eyes and then the rest of the pupa become melanized, and just prior to emergence a coal-black color is assumed. The sexes can be distinguished only by microscopic examination of the antennae. The pupal stage ranges from 11 to 16 days, with an average of about 13 days.

SEX RATIO AND NUMBER OF PARASITES PER HOST

From the original shipment from Japan, which consisted entirely of field-collected overwintering mummies, on an average 2.7 females to 1 male were obtained. Only 8.3 percent of the mummies produced an excess of males, while those yielding solely males or females were exceedingly scarce. In breeding experiments at Moorestown there were only twice as many females as males.

The material from Japan yielded 11.6 adult parasites per host, as compared with 5.2 from their progeny reared at Moorestown. The average mortality of mature larvae was also lower for the imported material—1.6 as compared with 4.3. It should be stated, however, that parasites received from Japan had been hand-picked and the imperfect and very small individuals removed. Furthermore, a portion of those propagated at Moorestown had entered the prepupal stage before being stored at 42° F. Since they overwinter as mature larvae, other stages cannot withstand low temperatures. It was also found that mealybugs parasitized in the third instar or in the

[9] HILL, C. C. PLATYGASTER HIEMALIS FORBES, A PARASITE OF THE HESSIAN FLY. Jour. Agr. Res. 32: 261–276, illus. 1926.

early adult female stage produced the greatest number of parasites. If parasitized prior to the third instar, many hosts become mummified before attaining full growth, while those parasitized as full-fed adult females often succeed in laying eggs and can mature few if any *Allotropa* larvae. Mealybugs parasitized in the first or second instar will frequently produce solitary mummies. The number of adult parasites issuing per host has ranged from 1 to 22.

Hosts of various instars were subjected to a single oviposition by different mated females and immediately dissected to ascertain the number of eggs laid. The number ranged from 1 to 18 eggs per host, with an average of 8.8, but did not seem to depend on the size of the host. Since the number of eggs deposited is approximately equal to the number of larvae that develop, it is obvious that monembryony is the rule.

LIFE CYCLE

At temperatures in the range of 75°–80° F. the life cycle generally runs as follows, although frequent variations are found, particularly in the postembryonic stages: Egg (including parasite body) 8.0 to 12.0 days, averaging 9.5 days; larva 5.5 to 7.5 days, averaging 6.5 days; prepupa 1.5 to 2.5 days, averaging 2.0 days; pupa 11.0 to 16.0 days, averaging 13.0 days. Since considerable growth occurs during embryonic development, the incubation period is proportionally long, while the larval feeding period is comparatively short and confined to a single instar.

Mummies containing mature *Alloptropa* larvae can be stored at low temperatures for many months. The material from Japan was collected in October 1939, and upon its receipt at Moorestown was stored at 42° F. According to samples incubated at frequent intervals, emergence was wholly normal until the following June—after at least 7 months under overwintering conditions.

SUMMARY

Allotropa burrelli Mues., a gregarious platygasterine parasite of *Pseudococcus comstocki* (Kuw.), was introduced into the United States from Japan in 1939. Propagation of stocks at the Moorestown, N. J., laboratory for release in infested apple orchards afforded an opportunity for a biological study of this parasite.

The adults are small and short lived, and oviposit at random in the host body cavity. There is no preoviposition period. All nymphal stages of mealybugs are attacked, but a preference is shown for those at least half grown. Dissections have disclosed on an average 565 eggs per female.

Development is normally monembryonic, although twinning may rarely occur. The parasite body increases to approximately 24 times its initial length during incubation, nourishment being elaborated by the trophamnion with its paranuclear masses. As development proceeds the parasite body becomes closely associated with the host fat body.

There is but one instar. A single pair of spiracles is located in segment 1, and there is no posterior commissure. The mouth parts are unusual, and there are 10 body segments. The food materials are

contained within a complete peritrophic sac. Supernumerary larvae and embryos are partially eliminated by cannibalistic attack.

The sex ratio has ranged from 2:1 to 3:1, with females predominating. Laboratory-reared material has produced from 1 to 22 parasites per host, with an average of 5.2. Mealybugs parasitized during the first or second stage frequently produce solitary mummies. An average of 8.8 eggs were deposited per host, regardless of size.

The life cycle ranges from 26 to 38 days, with an average of 31 days at 75°–80° F. Growth is most rapid during embryonic development, and the incubation period is nearly five times as long as the larval feeding period. Overwintering occurs as mature larvae, and mummies in that stage will endure prolonged cold storage.

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